ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Quick-RNA™ Plant Miniprep Kit

Catalog No. **R2024**

Highlights

- Quick, 10 minute isolation of inhibitor-free total RNA (~50 µg) from a wide variety of plant samples using ultra-high density *BashingBeads*™ and *Zymo-Spin*™ column technologies.
- High-quality RNA eluted in ≥50 µl is ready for reverse transcription, microarray, sequencing, etc.

Contents

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Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product please call 1-888-882-9682.

For assistance, contact us at tech@zymoresearch.com.

Product Contents

***Quick*-RNA™ Plant Miniprep Kit** (Kit Size)	**R2024** (50 preps.)
RNA Lysis Buffer	50 ml
RNA Prep Buffer	25 ml
RNA Wash Buffer[1] (concentrate)	24 ml
DNase/RNase-Free Water	4 ml
Prep Solution	30 ml
ZR BashingBead™ Lysis Tubes (2.0 mm)	50
Zymo-Spin™ IIICG Columns	50
Zymo-Spin™ IICR Columns	50
Zymo-Spin™ III-HRC Filters	50
Collection Tubes	2x 50
Instruction Manual	1

Note - Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

[1] Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate before use.

Specifications

- **Sample Types** – Leaves, stems, buds, flowers, fruit, seeds, etc.
- **Format** – Bead beating, spin column
- **Yield** – RNA can be eluted into ≥50 µl, allowing for a highly concentrated sample. Maximum RNA binding capacity of the provided column is ~50 µg.
- **RNA Purity** – High quality total RNA (A_{260}/A_{280} > 1.8, A_{260}/A_{230} > 1.8) is recovered. In general, traces of DNA may be present in the eluted RNA fraction. Complete removal of DNA can be accomplished by performing an in-column DNase I digestion (page 4).
- **Compatibility** – Compatible with samples stored in RNA*later* ™.
- **RNA Storage** - RNA is eluted with RNase-free water and can be stored at ≤-70 ºC. The addition of RNase inhibitors is optional but highly recommended for prolonged storage.
- **Required Equipment** – Microcentrifuge, vortex, cell disrupter/pulverizer (optional).

Product Description

The ***Quick*-RNA™ Plant Miniprep Kit** provides for rapid isolation of RNA from various plant samples (*e.g.* leaves, stems, buds, flowers, fruit, seeds *etc.*). For purification of total RNA including small RNAs (~50 µg), the ***Quick*-RNA™ Plant Miniprep Kit** features a specially formulated **RNA Lysis Buffer**. The **Zymo-Spin™ IIICG Column** allows for high-capacity DNA elimination and the subsequent **Zymo-Spin™ IICR Column** efficiently adsorbs total RNA.

The RNA is washed and then eluted with **DNase/RNase-Free Water**. For inhibitor removal, the eluted RNA can be treated by running the sample through the **Zymo-Spin™ III-HRC Filters**. The RNA is suitable for use in various subsequent procedures including RT-PCR.

The entire RNA isolation procedure typically takes about 10 minutes.

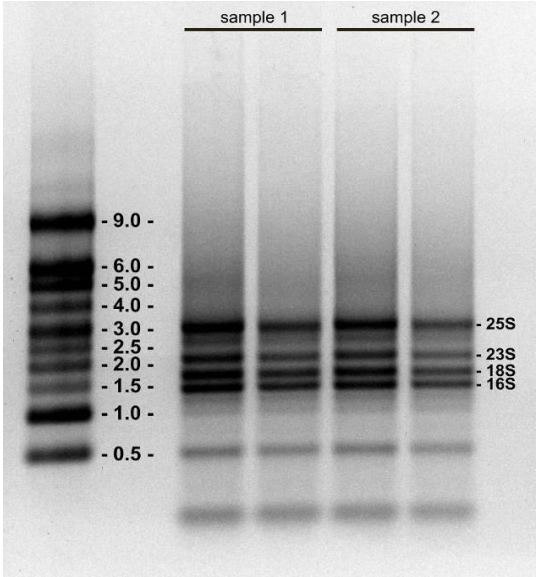

Figure 1:
Isolation of **total RNA** from **10 mg** of a fresh leaf (*Nicotiana sp.)* using the ***Quick*-RNA™ Plant Miniprep Kit**. Leaves were minced and processed with a FastPrep®-24 device *(MP Biomedicals*, see page 7). Samples 1 and 2 were loaded in 2x and 1x volume aliquots, respectively, and resolved in a 1% (w/v) nondenaturing agarose gel. RNA Millenium™ Markers *(Ambion)* were used as size standards.

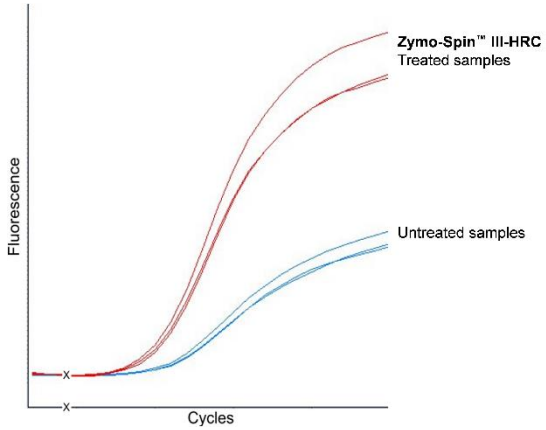

Figure 2:
Nicotiana sp. leaf samples were spiked with humic acid *(Sigma)* at a final Ab_{230nm} = 0.2. Total RNA was isolated with and without the use of the **Zymo-Spin™ III-HRC Filter**. RT-PCR performed with a LightCycler™ 480 *(Roche)* showed an increase in fluorescence signal and detected an early amplification initiation for the **Zymo-Spin™ III-HRC** treated samples compared to the non-treated samples (c_p = [30 vs. 31], respectively).

For **Assistance**, please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail tech@zymoresearch.com.

Note:

For isolation of inhibitor-free, PCR-quality DNA from variety of plant and seed samples see ***Quick*-DNA™ Plant/Seed Miniprep Kit** (Cat. **#D6020**).

Make sure guidelines are followed to ensure the RNA isolation procedure is performed in an RNase-free environment.

Notes:

[1] For example, up to 150 mg pre-cut leaves of *Nicotiana sp.*

[2] **Sample processing example:** *Nicotiana sp.,* fresh leaves: 6 m/s, 30-60 seconds – FastPrep®-24, or similar. See manufacturer's s literature for operating information.

[3] Sample *(i.e., supernatant)* and reagent volumes in this protocol can be adjusted proportionally if needed.

[4] To process samples >800 µl, reload the column.

[5] At this point, RNA samples can be in-column DNase I treated (page 4).

Reagent Preparation

✓ Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.

Protocol

Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

1. Collect fresh/frozen, finely minced plant sample[1] into a **ZR BashingBead™ Lysis Tube** and add 800 µl **RNA Lysis Buffer**.
2. Secure in a bead beater fitted with a 2 ml tube holder assembly and process[2].
3. Centrifuge the **ZR BashingBead™ Lysis Tube** for 1 minute.
4. Transfer 400 µl of the supernatant[3] into a **Zymo-Spin™ IIICG Column**[4] in a **Collection Tube** and centrifuge for 30 seconds. Save the flow-through!
5. Add 1 volume ethanol (95-100%) to the flow-through in the **Collection Tube** and mix well.
6. Transfer the mixture to a **Zymo-Spin™ IICR Column**[4] in a **Collection Tube** and centrifuge for 30 seconds[5]. Discard the flow-through.
7. Add 400 µl **RNA Prep Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
8. Add 700 µl **RNA Wash Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
9. Add 400 µl **RNA Wash Buffer** to the column and centrifuge for 2 minutes to ensure complete removal of the wash buffer. Transfer the column carefully into an RNase-free tube (not provided).
10. Add 50 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge for 30 seconds.
11. Place a **Zymo-Spin™ III-HRC Filter** in a new Collection Tube and add 600 µl **Prep Solution**. Centrifuge at 8,000 x *g* for 3 minutes.
12. Transfer the eluted RNA (step 10) into a prepared Zymo-Spin™ III-HRC Filter in an RNase-free tube (not provided) and centrifuge at exactly 16,000 *x g* for 3 minutes.

    The filtered RNA can be used immediately or stored at -70°C.

Appendix A: *In-Column* DNase Digestion

The DNase I digestion procedure can be performed using **DNase I Set** (E1010)[1].
Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

1. Following the RNA binding step (page 3, step 6), prewash the column with 400 µl **RNA Wash Buffer**. Centrifuge for 30 seconds. Discard the flow-through.

2. For each sample to be treated, prepare **DNase I Reaction Mix** in an RNase-free tube (not provided). Mix well by gentle inversion:

DNase I	5 µl
DNA Digestion Buffer	75 µl

3. Add 80 µl of the **DNase I Reaction Mix** directly to the column matrix. Incubate the column at room temperature (20-30°C) for 15 minutes. Then continue with RNA Purification (page 3, step 7).

Appendix B: Samples in DNA/RNA Shield™

1. Bring the lysed sample to at least 800 µl with **DNA/RNA Shield™**. Transfer the 800 µl sample to a **ZR BashingBead™ Lysis Tube**.

2. Secure in a bead beater fitted with a 2 ml tube holder assembly and process.

3. Centrifuge the **ZR BashingBead™ Lysis Tube** for 1 minute.

4. Transfer 400 µl supernatant to a new tube (not provided).

5. Add 1 volume **RNA Lysis Buffer** to the sample and mix well.

6. Transfer the mixture to a **Zymo-Spin™ IIICG Column** in a **Collection Tube** and centrifuge for 30 seconds. Save the flow-through!

7. Continue with RNA Purification (page 3, step 5).

Notes:

[1] Prior to use, reconstitute the lyophilized **DNase I** as indicated on the vial. Store frozen aliquots.

Unit definition - one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A_{260} units/min/ml of reaction mixture at 25°C.

Ordering Information

Product Description	Kit Size	Catalog No.
***Quick*-RNA™ Plant Miniprep Kit**	50 Preps.	R2024

For Individual Sale	Amount	Catalog No.
ZR BashingBead™ Lysis Tubes (2.0 mm)	50	S6003-50
RNA Lysis Buffer	50 ml 100 ml	R1060-1-50 R1060-1-100
RNA Prep Buffer	10 ml 25 ml	R1060-2-10 R1060-2-25
RNA Wash Buffer (concentrate)	6 ml 12 ml 24 ml 48 ml	R1003-3-6 R1003-3-12 R1003-3-24 R1003-3-48
Zymo-Spin™ IICR Columns	50 250	C1078-50 C1078-250
Zymo-Spin™ IIICG Columns	50 250	C1006-50-G C1006-250-G
OneStep™ PCR Inhibitor Removal Kit	50	D6030
Collection Tubes	50 500 1000	C1001-50 C1001-500 C1001-1000
DNase/RNase-Free Water	1 ml 4 ml 6 ml 10 ml	W1001-1 W1001-4 W1001-6 W1001-10

Description	Amount	Cat. No.
Disruptor Genie™, 120V w/ 2 ml tube holder assembly.	1 unit	S6001-2-120
Disruptor Genie™, 240V w/ 2 ml tube holder assembly.	1 unit	S6001-2-240
TurboMix Attachment, 2 ml Permanently mounts to most existing Vortex Genie™ mixers converting them to a Disruptor Genie™.	1 unit	S6004-2

The **Disruptor Genie™** with 2 ml tube holder from Scientific Industries, Inc. (Cat. No. S6001-2 from Zymo Research Corp.)

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